

REVIEW

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Controlling the inflammatory response through transcriptional mechanisms

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Introduction

The central feature of the immune system is the ability to distinguish foreign antigens from the proteins of the organism itself and to mount appropriate responses to them. The type of immune response depends in part upon the nature of the antigen, but it broadly falls into one of two distinct classes. These two responses are affected by distinct populations of CD4⁺ lymphocytes termed Th1 and Th2, which are derived from a common precursor. Cell-mediated responses require that peptide antigens from intracellular pathogens and viruses are displayed on the surface of an antigen presenting cell in the context of MHC class I or II proteins in order to be recognized by the T cell receptor of Th1 lymphocytes. In contrast, humoral immunity is mediated by B cells, which require signals from Th2 lymphocytes in order to secrete immunoglobulins that are involved in allergic responses and eradication of extracellular parasites [1,2]. The two cell types display specific patterns of cytokine expression with Interleukins (IL)-4, -13 and -5 being expressed by Th2 cells and interferon- γ (IFN γ) by Th1.

Asthma is characterized by reversible airways obstruction and eosinophilia coupled with bronchial hyperresponsiveness to a variety of specific and nonspecific stimuli. The pathophysiology of asthma is mediated by overproduction of Th2 cytokines. The genes for IL-4, -5 and -13 are located in close proximity on human chromosome 5q31 [3]. Eosinophilia is in part caused by IL-5 release, whereas IL-4 and IL-13 evoke production of IgE. As a consequence, attention has focused on the specific transcriptional

mechanisms controlling cytokine expression. Reverse genetics and the generation of mutant mouse strains has served to identify factors playing important roles in the restrictions generating the individual cell types (Reviewed in [4–6]), and also in establishment of cell type-specific cytokine expression. The absolute levels of cytokines produced are controlled by signals acting to upregulate ubiquitous factors such as the AP-1 complex, NFAT and NF κ B. It is primarily at the level of activated transcription that therapeutic agents such as glucocorticoids function, and a fuller understanding of transcriptional control of Th2 cytokine genes may result in novel therapeutic strategies. In this review, we discuss some of the key proteins involved in the establishment of Th1 and Th2 lineages and in tissue-specific expression of cytokine genes. Furthermore we discuss factors which cause upregulation of these genes, and potential mechanisms of glucocorticoid regulation.

T cell differentiation

Two critical features of T cell differentiation are the ability to rearrange DNA at the T cell receptor (TCR) loci to generate a wide variety of variable regions of the receptor. This provides diversity of antigen recognition. A second feature is clonal proliferation of cells bearing a given receptor upon encounter of a specific antigen generating a concentrated antigenic response. T cells are able to produce two general classes of TCR, $\alpha\beta$ and $\gamma\delta$, but by the time of birth in mice, the $\alpha\beta$ is the predominant subtype. $\alpha\beta$ T cells can form either the cytotoxic lineage, which express the CD8 coreceptor, and have capacity to lyse cells, or the helper subtype, which express CD4, and can assist either T-cell mediated killing or B-cell-mediated immunoglobulin synthesis.

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T cells migrate throughout the blood and lymph system until they encounter a specific peptide antigen bound to a MHC molecule on an antigen-presenting cell. This causes engagement of the TCR and a second costimulatory pathway, the most important of these pathways is mediated by CD28 [7]. Much of the co-operative interaction between TCR and CD28 is facilitated by the induction of NF κ B, NFAT and AP-1 family members. Increases both in quantity and in activity of these families has been demonstrated [6,8]. Activation of T cells causes the regulated transient expression of over 100 genes including pro-inflammatory cytokines, chemokines, inflammatory enzymes, adhesion molecules and receptors which form a feedback cascade.

Some of the earliest differentiative events include expression of transcription factors either involved in expression of the TCR subunits, or regulating chromatin structure at these loci making them competent for transcription. Subsequent lineage development follows locus rearrangement and the naive T cells encountering antigen in the peripheral lymphoid organs. However, generation of effector proteins and cytokines from these naïve cells usually requires a number of cycles of cell division.

T helper cell differentiation

The Stat family of transcription factors are known to be important mediators of cytokine-induced signalling [9,10]. Development of murine Th1 cells is driven by IL-12 produced by macrophages and dendritic cells. This cytokine, acting through Stat4, stimulates Th1 cells to produce IFN γ , which in turn upregulates expression of the IL-12 receptor, thereby potentiating commitment. Stat4 is phosphorylated and becomes transcriptionally active upon stimulation of CD4⁺ naive cells with IL-12, and Stat4-deficient mice demonstrate severe defects in Th1-mediated immune responses, including decreased IFN γ production, and enhanced development of Th2 cells [11,12]. Th2 differentiation, in contrast, is stimulated by IL-4. Stat6 is activated via engagement of the IL-4 receptor (IL-4R) and causes the activation of a variety of IL-4-regulated genes, such as IL-4R and IgE [6]. The role of Stat6 in the development of Th2 cells was revealed by production of Stat6-deficient mice [12–14]. These mice failed to develop Th2 responses, such as IgE class-switching and naive T-cells failed to differentiate into Th2 cells upon *in vitro* stimulation. Furthermore, synthesis of IL-4 and IL-5 was markedly diminished. Similar subsets of Th cells have also been described for humans [15]. More recent work has suggested, however, that the Th1/Th2 phenotypes initially described may be polarized examples of the more heterogeneous patterns of expression observed *in vivo* [1,16]. Other subsets of CD4⁺ T cells have recently been described such as

the Tr1 subset which expresses high levels of IL-5 and IL-10 but no IL-4 and low levels of IL-2 [17]. Even so, there seems little doubt that skewing of the Th cell cytokine profile performs an important role in determining the susceptibility to pathogenic infections and allergy. It is thought that some of the effector functions of IL-13 and IL-4 overlap. Both cytokines are able to induce isotype class switching in B cells to IgE. IL-13 has been suggested to be the central mediator of allergic responses and asthma [18,19]. The function of IL-5, in contrast, appears to be focused on eosinophils, effecting differentiation, proliferation, activation and survival.

A number of proteins have been postulated as controlling the differentiation of naive T cells into the Th1 and Th2 lineages. Proteins implicated in Th2-specific cytokine expression are GATA3, c-Maf, JunB and C/EBP β , while recently, T-bet has been demonstrated to be involved in regulating Th1 differentiation (Fig. 1).

GATA3

Numerous data support a role for GATA3 in Th2 development, differentiation and activation. Ting and colleagues demonstrated using the Rag deficient blastocyst complementation assay, that mice developed from GATA3^{-/-} embryonic stem cells injected into Rag 2^{-/-} blastocysts were completely devoid of T cells [20]. The essential role in T-cell development is supported by the fact that GATA3 is involved in transcriptional regulation of the α , β and δ TCR chains [21–23]. Flavell and colleagues have demonstrated that GATA3 is expressed in both naïve and Th2 lineage cells but expression is extinguished in Th1 cells [24]. GATA3 has been shown to have a role in transcriptional regulation of the IL-5 gene through direct interaction with sites within the proximal promoter [25,26]. There has been no direct evidence of the ability of GATA3 to bind to the IL-4 proximal promoter, however, expression of antisense GATA3 message in T cells caused a reduction in secreted IL-4 levels [24,26]. Ho and colleagues have suggested that 800 bp of the IL-4 promoter is sufficient to confer GATA3 responsiveness [27,28]. In addition to its role in induction of the Th2 lineage, Murphy and colleagues have recently suggested that GATA3 plays a role in inhibition of Th1 development, which does not require IL-4. GATA3 represses IL-12 receptor β 2 mRNA expression thereby preventing IL-12 signalling and consequently its positive effect in propagation of the Th1 lineage [29].

c-Maf

c-Maf was isolated from a yeast two hybrid screen for proteins that interact with NFATp, and was shown to transcriptionally activate the IL-4 promoter [30]. Whereas the data supporting a role for GATA3 in transcriptional

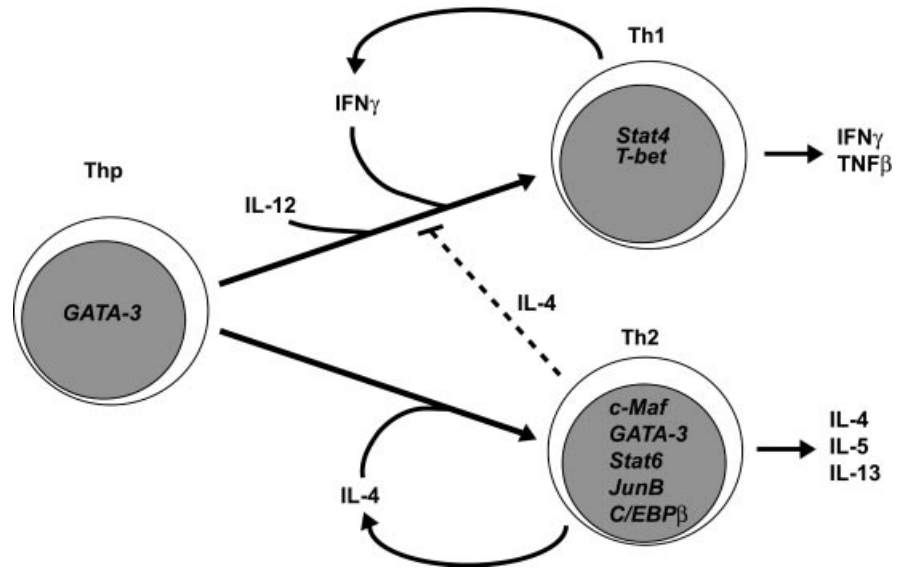


Fig. 1. Factors influencing Th1/Th2 differentiation. Transcription factors are shown in *italics*. Dotted line indicates negative effect of IL-4 on Th1 differentiation. Thp: T helper precursor (naive T cell).

regulation of IL-4 in T cells is inconsistent, c-Maf has been shown to bind directly to the IL-4 promoter through a Maf response element (MARE) located in the proximal promoter [30,31]. This region is known to be important for the Th2-specific expression of IL-4. c-Maf is expressed in numerous tissues including kidney, testis, placenta and skeletal muscle. Within the lymphoid system it is upregulated during Th2 differentiation but is not expressed in Th1 cells. Expression of c-Maf, NFATp and the NFAT-interacting protein NIP45 is sufficient to activate the endogenous IL-4 promoter in HepG2 liver cells [31]. c-Maf $(-/-)$ mice have recently been generated, disruption affects both intrauterine and postnatal survival with death occurring at E 17.5–18.5. c-Maf $-/-$ mice also develop microphthalmia beginning approximately two weeks after birth [28]. Spleen cells from c-Maf $-/-$ mice produce virtually no IL-4, but other cytokines including IL-5, IL-6 and IL-10 are present in these cultures albeit at reduced levels. In contrast, IL-13 levels are normal, as are IgE levels [32]. This suggests both that IL-13 is capable of inducing isotype switching to IgE in B cells. Recent data from Brown and colleagues [33] have demonstrated that mast cells, which, along with basophils, can also generate IL-4, do not express c-Maf, and suggest that the Maf response element located in the IL-4 proximal promoter is not critical for promoter function in these cells. c-Maf expression in Th2 cells is maximal 8 days after T cell activation [30], when differentiation has been completed for some days. It is possible that low level expression is sufficient for activation of IL-4 transcription, alternatively, the source of IL-4 necessary to initiate Th2 lineage development from the naive precursor is derived from a different cell type, and only subsequently is c-Maf activated to generate sufficient cytokine to cause Th-2 proliferation.

JunB

A potential role for AP-1 proteins during T-helper cell differentiation was suggested by Flavell and co-workers [34] who demonstrated that a high level of AP-1 activity was induced upon stimulation of Th2 but not Th1 cells. This activity correlated with accumulation of JunB in Th2 cells but not Th1 or Th0 cells. It has been demonstrated that the P1 site within the proximal IL-4 promoter, which binds NFAT/AP-1 complexes, drives Th2-restricted expression. JunB has been postulated as providing the specificity. In transgenic mice, JunB overexpression causes upregulation, IL-4, IL-5, IL-6 and IL-10, although it is unclear whether the effects on IL-5, -6 and -10 is direct or secondary to IL-4. JunB synergises with c-Maf to activate IL-4 expression, and its activity is stimulated following phosphorylation by Jun N-terminal kinase [35].

C/EBPβ

C/EBPβ (NF-IL-6) has been shown to transcriptionally activate the IL-4 promoter [36], but its expression is not limited to Th2 cells. C/EBPβ binds weakly to both an NFAT/AP-1 site, and a MARE within the IL-4 promoter. Mutation of these sites within the IL-4 promoter abrogates transactivation in response to numerous stimuli. Krammer and colleagues have additionally suggested that C/EBPβ, may not be expressed in Th1 clones [37].

T-bet

While the study of Th2 differentiation has revealed a number of candidate factors involved in expression of IL-4 and -5, until recently, little was known about proteins

driving expression of Th1 cytokines such as IFN γ . Recently however, Glimcher and colleagues have reported cloning of a T-box factor T-bet whose expression correlates with IFN γ production [38]. Ectopic expression of T-bet is sufficient to activate endogenous IFN γ . Notably, T-bet expression in polarized Th2 effector cells causes reprogramming to a Th1 phenotype, repression of IL-4 and IL-5 production and expression of IFN γ , although T-bet did not repress transcription of an IL-4 promoter driven reporter construct. IL-5 production was more sensitive than IL-4 to T-bet expression suggesting a potential for regulation of GATA3 activity, and a potential coregulatory activity which determines whether a Th1 or Th2 fate is adopted.

Chromatin structure

Much of the data that has accumulated on expression of the individual cytokines has revolved around factors binding to the promoter regions of these genes. Numerous recent studies, however, have amply demonstrated the importance of chromatin structure at gene loci in transcriptional regulation. Agarwal & Rao have postulated that during differentiation to the Th2 lineage, there is progression towards an 'open' chromatin structure at the 5q31 locus as demonstrated by the appearance of several DNaseI hypersensitive sites around the IL-4 gene [39]. In progression to the Th1 lineage, the IFN γ locus is remodelled. Both of these changes are accompanied by CpG demethylation. Histone acetylation and deacetylation has been the subject of intense recent investigation since the two events provide a direct mechanism by which cofactors for DNA-binding transcription factors can effect the interaction of core histones with DNA. Acetyl transferases act to weaken histone DNA interactions, thereby making DNA available to the transcriptional machinery (Fig. 2), whereas deacetylases reverse this process. There has been a great deal of speculation regarding which factors mediate remodelling, GATA3 in particular has been suggested to fulfil this function in Th2 cells. It is expressed early in T-cell differentiation and is structurally highly conserved with GATA1, which associates with, and is acetylated by the histone acetyl transferase (HAT) CBP [40,41]. To date, however, there is no data to support such an activity. The list of transcription factors that have been demonstrated to interact with coactivators or corepressors with HAT or deacetylase activity, respectively, has, however, grown exponentially. Both NFAT and AP-1 proteins have been shown to functionally interact with HAT's [42,43], and it is an emerging theme that coactivator recruitment is a common mechanism of transcription factor function.

Co-ordinated expression of the 5q31 cytokines, despite transcriptional regulation by diverse factors remains a feature of Th2 cells. The recent demonstration of a 400-bp

element between the IL-4 and IL-13 genes, whose deletion in YAC transgenic mice causes a decrease in numbers of T cells expressing IL-4, IL-5 and IL-13 suggests there may be an, as yet unidentified, global regulator of these genes [44]. It is suggested that this element contains no consensus binding site for GATA3, c-Maf, NFAT or Stat6, although binding to nonconsensus sites has not been excluded. IL-4 is expressed monoallelically [45,46], no data has yet been presented as to whether this is also true for IL-13 and IL-5.

Activation and steroid regulation

The cytokine genes are responsive to activation, brought about by the recognition of specific antigens presented by antigen presenting cells (APC's). This activity, mediated by the T cell receptor, enhances intracellular calcium levels and protein kinase C activity. The hallmark of activation is IL-2 production, IL-2 mediates the clonal expansion of T cells whereas cells from mice lacking IL-2 proliferate poorly [47]. Three classes of transcription factors have been shown to have a key role in mediating activation signals. These are NFAT, NF κ B and AP-1, which have been shown to be widely involved in regulation of transcription of cytokines, chemokines, inflammatory enzymes, adhesion molecules and cell surface receptors. Consequently their activity serves both to amplify and perpetuate the inflammatory response. Much data has been accumulated showing that both NF κ B and AP-1 are targets for glucocorticoids.

AP-1

The AP-1 family of proteins regulate transcription of many cytokines including both IL-4 and IL-5 [48,49]. c-Fos and c-Jun are the prototypes of the family of proteins (FosB, Fra1 and Fra2; and JunD and JunB) which bind to the AP-1 site or TRE (12-o-tetradecanoyl phorbol-13-acetate response element). The Fos and Jun families both possess DNA binding domains of the basic domain-leucine zipper (b-zip) family which allow dimerization as a prerequisite to DNA binding. The association is usually as a heterodimer formed between individual subunits of the two families, although Jun proteins may homodimerize. c-Jun and c-Fos both possess transcriptional activation domains, one of which shares a common motif (HOB1) [50]. In c-Fos HOB1 is stimulated by phosphorylation by the MAP kinase family. c-Jun transcriptional activity is enhanced by phosphorylation of serines 63 and 73 within its amino terminus [51,52]. This phosphorylation is mediated by the Jun N-terminal kinase family (JNK 1-3) also known as the stress activated protein kinases SAPK's [53,54]. The ability of the adenoviral protein E1A to repress activity of the collagenase promoter via an AP-1 site despite an inability to affect DNA binding capacity of the AP-1 complex led to

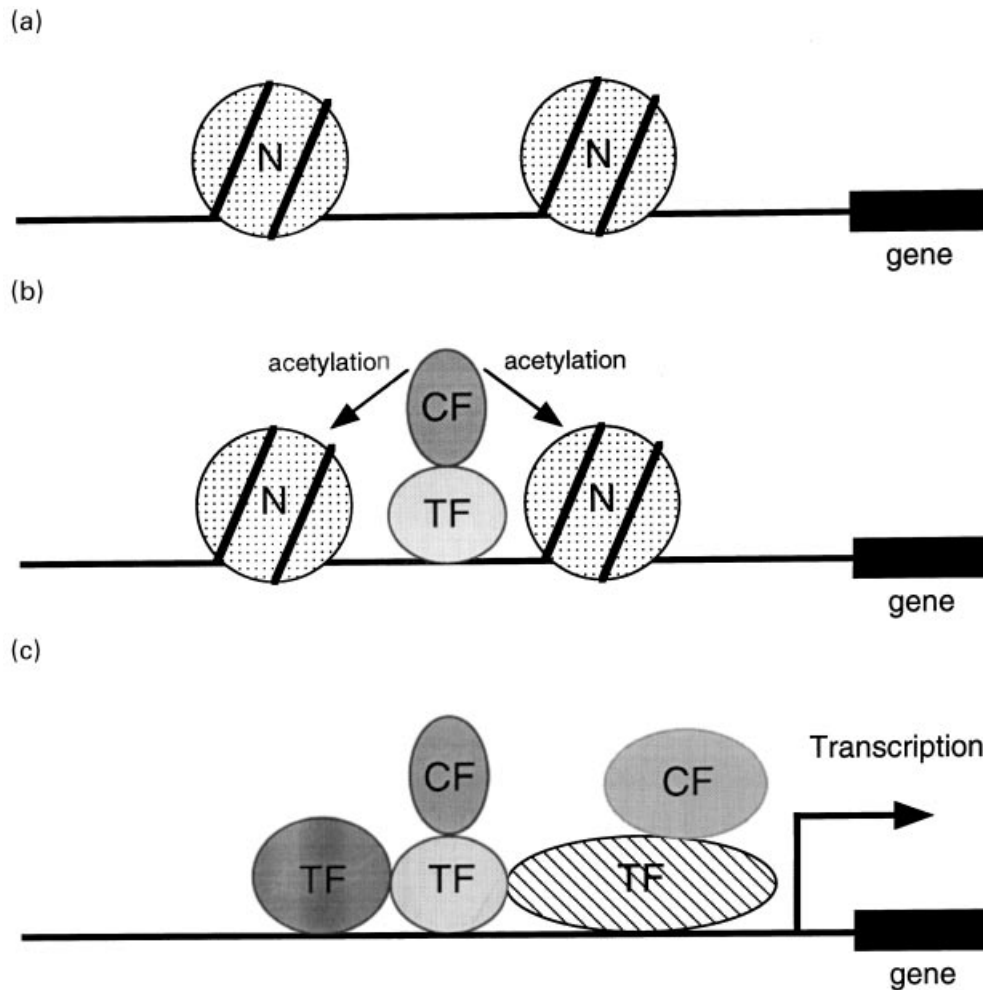


Fig. 2. Chromatin remodelling in transcriptional regulation. (a) DNA exists in a highly compact structure which is not permissive for transcription. It is wrapped twice around the histone octamer to form nucleosomes (N). DNA binding by certain transcription factors (TF) allows recruitment of cofactors (CF), some of which have histone acetyl transferase activity. (b) Acetylation of specific histone N-terminal lysine residues makes the interaction with the DNA backbone weaker, allowing dissociation of DNA from the nucleosome. (c) Following dissociation DNA is available for binding by other transcription factors, allowing recruitment of RNA polymerase and transcriptional activation.

the postulate that AP-1 transcriptional activation was mediated by an adapter protein. This protein has subsequently been shown to be CBP/p300 [55,56]. CBP fits the criterion of a coactivator since it bridges transcriptional activators such as Jun and the basal transcriptional machinery by a direct contact with TFIIB, and has inherent HAT activity [57]. Fos can bind CBP directly in an unphosphorylated form, and this interaction enhances the transcriptional activity of Fos [58]. CBP also binds to and transactivates c-Jun. The interaction take place within the N-terminal activation domain of Jun. Mutation of serines 63 and 73 in c-Jun reduces CBP binding *in vitro* and CBP-dependent transactivation. The initial observations of a capacity of the glucocorticoid receptor to regulate AP-1 activity detailed that an interaction took place between GR

and c-Fos that did not require DNA binding by either protein, reducing binding to the AP-1 site [59–61]. In other studies, repression has not been consistent with loss of DNA binding as demonstrated by DNA footprinting. It has been suggested that the repressive mechanism may be heterogeneous, since in other systems, GR has been demonstrated to repress AP-1 activity while AP-1 is bound to the TRE [62]. A similar function is thought to be involved in the repression of the IL-2 enhancer in which GR represses activation mediated by AP-1 and NFAT. The AP-1 site confers GR sensitivity [63], repression being caused by prevention of the NFAT/AP-1 interaction [64].

It is now apparent that the repressive activity of GR occurs at numerous points within a signalling cascade. Caelles *et al.* have demonstrated that hormone activated

GR inhibits the induction of the JNK signal transduction pathway and therefore interferes with c-Jun phosphorylation that is required for CBP interaction [65]. JNK also mediates phosphorylation, and therefore activity of Elk1, which is a transcriptional activator of c-fos [66]. AP-1 inhibiting activity by nuclear hormone receptors was initially described for gr; however, other family members such as RAR, T3R, VDR and RXR possess the same activity [67].

NFκB

NFκB was initially described as a complex able to bind to and transactivate the kappa light chain enhancer [68] and is a member of the Rel family. The prototypical NFκB is a heterodimer of p65 and p50 subunits [69], of which RelA (p65) stimulates transcription of diverse genes. Other subunits exist, such as rel, relB and p52. It is likely that different heterodimers have both specific target genes and functions. NFκB activity is affected by a wide variety of stimuli including cytokines such as Interleukins -1, -2, -6 and -8, growth factors such as GM-CSF, oxidants, phorbol esters, T-cell receptor ligation and allergens, and bacteria and viruses (reviewed in [70]). NFκB has a widespread role in activating transcription of genes involved in the inflammatory process, including those for the cytokines IL-1β, IL-2, IL6 and IL-3, and the growth factor GM-CSF. Chemokines such as IL-8 and eotaxin, adhesion molecules such as ICAM and VCAM and receptors such as the IL-2 receptor, the β chain of the T cell receptor and the platelet activating factor receptor, these genes are negatively regulated by glucocorticoids. Overall there is significant overlap between inflammatory genes regulated by NFκB and proteins that activate NFκB.

NFκB forms a model for transcription factors that are compartmentalized within the cell, this is achieved by complex formation with proteins of the IκB family, which act to target NFκB to the cytoplasm. Phosphorylation of IκB causes its dissociation from NFκB, ubiquitination and degradation within the proteasome. This results in free NFκB, which then translocates to the cell nucleus [71]. Five IκB isoforms are present within the cell although the alpha and beta subtypes are most prevalent (reviewed in [72]). Their structure contains ankyrin repeats, which bind to, and mask, the nuclear localization signal on NFκB thereby preventing its access to the nucleus. The IκB genes show differences in their transcriptional regulation. IκBα transcription is stimulated by both glucocorticoids and NFκB, it thereby acts as a regulator of NFκB activity since it exports NFκB back from the nucleus, and thus its target genes, to the cytoplasm. The IκB epsilon promoter also has NFκB sites [73]. IκBα -/- mice die of widespread inflammation presumably because NFκB becomes consti-

tutively active due to its retained nuclear localization; it appears therefore that the other IκB family members are unable to functionally complement this defect. In contrast IκBβ is not transcriptionally stimulated by NFκB. As with AP-1, there is also a direct interaction of GR with NFκB, which prevents binding of NFκB to its target sites [74,75].

The phosphorylation of the IκB family is central to regulation of NFκB function and has been seen to be an important focal point for therapeutic intervention. All of the activators of NFκB function by inducing IκB degradation by phosphorylation [76]. Because of the numerous diverse activators of NFκB activity, it appears that a variety of signalling cascades can converge at the level of IκB phosphorylation. Some of the kinases involved have been identified, such as the serine/threonine kinase NIK, and CHUK, another serine/threonine kinase, which acts downstream of NIK. IκB kinase (IκK) is highly homologous to CHUK and phosphorylates the critical serines 32 and 36 on the IκB molecule in response to cytokines [71]. The consequences of activating NFκB vary dependent upon the source of the stimulus and the influence upon the IκB family. TNFα stimulation causes a rapid degradation of IκBα but not IκBβ [77]; it also activates transcription of NFκB and consequently indirectly stimulates *de novo* transcription of IκBα. This protein then sequesters NFκB and exports it from the nucleus. The activation of NFκB is therefore transient. Interleukin 1β activation causes both IκBα and IκBβ to be degraded. Upon re-synthesis, IκBβ is hyperphosphorylated, interaction with NFκB leaves the NLS exposed resulting in localization of the NFκB/IκBβ complex to the nucleus and consequently a prolonged expression of genes regulated by NFκB. Transcriptional activation by NFκB involves recruitment of a coactivator complex that is similar to that recruited by nuclear receptors, and includes CBP/p300, members of the p160 coactivators, and pCAF [78,79].

NFAT

NFAT was first identified as a complex that bound to an activation responsive element in the IL-2 promoter upon T-cell stimulation [80]. Subsequently NFAT sites have been described in the promoters of IL-4, IL-5 and GM-CSF. The NFAT complex contains a nuclear component that is synthesized upon activation and a pre-existing cytoplasmic component that translocates to the nucleus upon activation. The nuclear component consists of several AP-1 family members including Fra-1 and JunB, the cytoplasmic component was identified as the transcription factor NFAT [80,81]. It is now clear that there are at least four different transcription factors of the NFAT family, all of which are widely expressed. NFAT3 does not appear to be expressed in T-cells. The fact that NFAT1, NFAT2 and NFAT4 are

all expressed in T cells, and that there are several isoforms of each protein, has made it difficult to elucidate the precise role of NFAT in T cell activation and Th1/Th2 differentiation [80,82,83]. Recent progress has been made, however, using transgenic mice lacking individual NFAT family members. Deletion of NFAT1 results in hyperproliferation of both B and T cells [31,84]. T cells from these mice demonstrate defective IL-4 production early in an immune response but also show an enhanced Th2 response at later timepoints, suggesting that NFAT1 performs a negative regulatory role in Th2 development. Disruption of NFAT2 causes death *in utero* due to circulatory failure because of defective cardiac valve formation [85,86]. The role of NFAT2 in T-cell regulation was studied using RAG1- and RAG2-deficient blastocyst complementation [86,87]. These experiments demonstrated that NFAT2-deficient mice have reduced numbers of thymocytes, impaired proliferation of T cells and a profound defect in Th2 responses, suggesting that it performs an activating role in Th2 development. NFAT4-deficient mice demonstrate increased levels of apoptosis in DP thymocytes which causes impaired development of CD4⁺ and CD8⁺ T cells which are hyperactivated [88]. Mice deficient in both NFAT1 and NFAT4 develop a profound lymphoproliferative disorder, increased IgG1 and IgE and a selective increase in Th2 cytokines [86]. It appears that the different NFAT family members perform antagonistic roles during Th1/Th2 development, NFAT1 and 4 appear to suppress Th2 cytokines, whereas NFAT2 induces Th2 development. The precise mechanisms by which these different functions are achieved remain to be determined, however, a recent paper has demonstrated that during T-cell activation NFAT2 activity is regulated by the transcriptional coactivator CBP/p300 [42].

Glucocorticoid regulation

Glucocorticoids are one of the mainstays of therapeutic intervention in asthma and allergy, resulting in reduced eosinophilia and promotion of eosinophil and T-cell apoptosis and proliferation. The hormone diffuses into the cell and interacts with the glucocorticoid receptor, freeing it from its cytoplasmic tethers and allowing nuclear localization. The structural organization of GR was resolved by a series of mutagenesis experiments in the mid 1980s [89,90]. This delineated regions of the protein required for ligand binding, dimerization, DNA binding and transactivation. Following cloning of the receptor, target genes were identified, such as the human metallothionein gene, and the glucocorticoid response element (GRE) was subsequently identified as a palindromic sequence composed of two 6 bp half sites, separated by a 3-bp spacer. These simple GRE's are defined as sequences that have no

effect on basal promoter activity in the absence of hormone or receptor, they do not mediate repression, and confer enhancement in a manner that simply tethers receptor to DNA.

In contrast to the established activation function of GR, certain genes are transcriptionally repressed by glucocorticoid hormones. These include pro-opiomelanocortin (POMC), collagenase, and the α -subunit of glycoprotein hormones. Analysis of their promoters has revealed novel aspects of glucocorticoid receptor function. Studies of the collagenase promoter have shown that GR is able to bind directly to components of the AP-1 transcription factor and in doing so, prevent access to their binding site, the TRE, consequently preventing transcriptional activation [59,60]. Within the α -subunit promoter a second mechanism for repression was postulated. In this circumstance, overlapping binding sites exist within the promoter for the cAMP responsive transcription factor (CREB) and for GR. Binding of GR to its site interferes with CREB binding and therefore prevents activation [91]. The pro-opiomelanocortin gene (POMC) harbours a negative GRE (nGRE) which, *in vitro* has been shown to support binding of a GR trimer. Here it is thought that GR may prevent interactions between factors binding to upstream elements [92].

A third class of binding site, the composite GRE has also been proposed. At these sites, such as within the proliferin promoter, GR interacts with specific DNA sequences and with DNA bound nonreceptor factors [62]. Although GR can bind DNA in the absence of the nonreceptor proteins, it only regulates transcription in their presence and may either activate or repress transcription depending upon the composition of the nonreceptor components, or the cell type. Within the proliferin GRE (plfG), AP-1 components act as selectors for changes in regulatory activity such that transcriptional activation in response to GR in the presence of c-Jun was switched to repression when fos protein was additionally present [62].

In addition to regulation of activity following ligand binding, the glucocorticoid receptor is phosphorylated in the amino-terminal transcriptional regulatory region [93] by Jun N-terminal kinase. This results in an inhibition of GR-dependant transcriptional enhancement, the cause of which has been postulated to be either an alteration in nuclear transport, or an altered affinity for coactivators [94]. Support for this suggestion has been provided by Okret and colleagues who have suggested that the interaction of GR with RIP140 antagonized all GR-mediated responses [95]. Similar regulation of function of nuclear hormone receptors has previously been described for SF1 [96], and the oestrogen receptor [97]. Further mechanisms allowing regulation of repression by nuclear hormone receptors involves the targeted degradation of corepressors as has been described for N-CoR. The murine

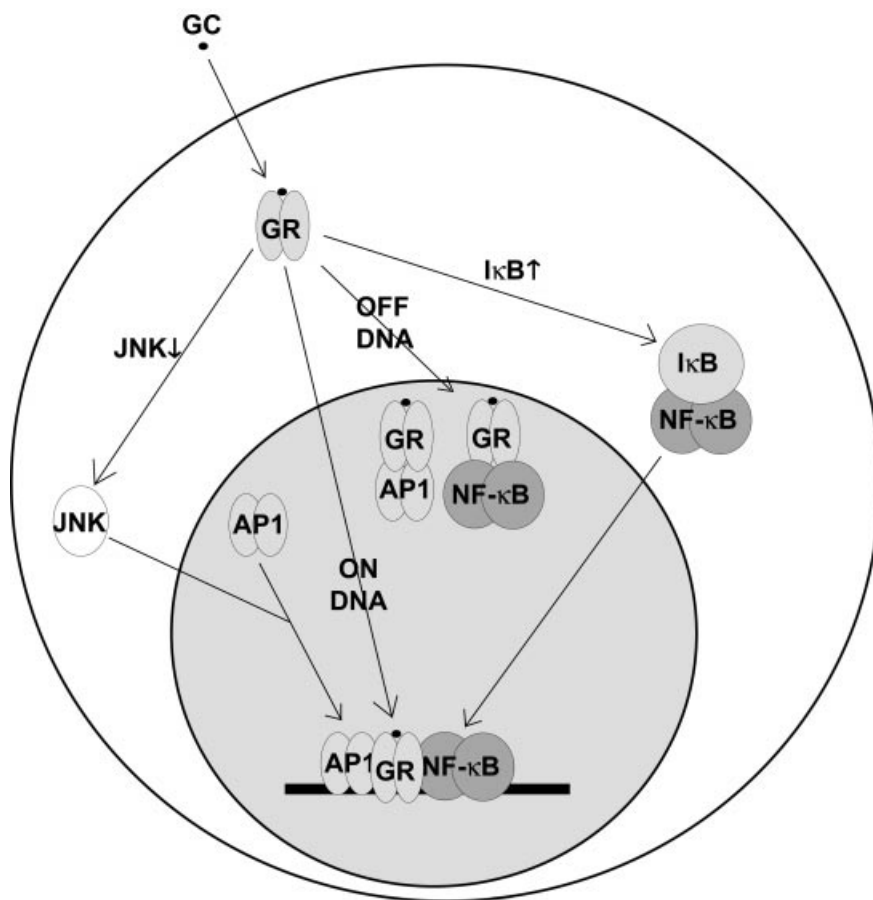


Fig. 3. Mechanisms of glucocorticoid action. Established targets and subcellular localization of events controlling transcriptional regulation by the glucocorticoid receptor. GC: glucocorticoid. GR: glucocorticoid receptor. JNK: Jun N-terminal kinase. AP-1: Activating Protein 1. NFκB: Nuclear Factor of κ light chain enhancer. IκB: Inhibitor of κB.

homologue of the *drosophila* protein seven in absentia (mSiah2) interacts with N-CoR, targets it for destruction and therefore acts as an antirepressor [98].

The GR is subject to complex regulation. Cytoplasmic retention in the absence of ligand suggests that the exchange of cofactors upon ligand binding that is observed for other nuclear hormone receptors may not take place [99]. There has so far been no demonstration of an interaction between GR and a corepressor with deacetylase activity, however, since GR is known to repress transcription, such an interaction is not unlikely. In this circumstance it might be argued that interaction with other transcription factors, GR modification by phosphorylation, or allosteric effects of GR binding to nonconsensus sites might alter affinity for cofactors. Radical data have recently been reported by Reichardt and colleagues on the relative importance of on and off DNA functions of GR. Using a knock-in model, a region of the GR previously shown to be critical for dimerization has been mutated to yield a dimerization deficient receptor [100]. Remarkably, mice homozygous for this mutant are both viable and fertile. Although any functions mediated by GR binding DNA as a monomer cannot be assessed in this system, the data imply

that binding to DNA as a homodimer and therefore functions mediated by the GRE are dispensable for survival. Thus, provided the animal can survive stress, the most important role for GR is in transcriptional interference.

Glucocorticoid-insensitive asthma

In view of the reciprocal interactions between AP-1 and GR, recent work has addressed the hypothesis that there may be dysregulation of AP-1 control in glucocorticoid-insensitive bronchial asthma. The activities of AP-1, NFκB and CREB in unstimulated PBMC's from glucocorticoid-resistant and -sensitive subjects were studied in gel retardation assays. These studies have suggested increased AP-1 DNA binding in the resistant subjects [101]. T lymphocytes and monocytes from resistant subjects generate a twofold excess of c-Fos protein relative to those with a glucocorticoid-sensitive phenotype [102]. The resulting increased AP-1 activity may render glucocorticoids less effective through reciprocal negative transcriptional interference. Inducible AP-1 is found after activation of specific mitogen-activated protein kinases (MAPK's), of which the Jun N-terminal kinase (JNK) family are a central

component [103]. JNK's increase AP-1 gene transactivation capacity by enhancing the affinity of phosphorylated c-Jun for c-Fos. Recent data has shown that the mononuclear cells of glucocorticoid resistant asthma subjects have increased expression of c-Fos, phosphorylated c-Jun and phosphorylated JNK when compared to glucocorticoid sensitive subjects [104]. Glucocorticoids suppressed JNK activity in glucocorticoid sensitive but not resistant subjects suggesting that increased JNK may be one mechanism central to pathophysiology of glucocorticoid-resistant asthma.

Summary and conclusions

In this review, we have suggested ways that GR can repress transcription of cytokine genes (Fig. 3). Both NF κ B and AP-1 may be targeted directly by an off DNA interaction. GR also possesses a capacity to bind to composite AP-1 sites. Furthermore, GR represses JNK-mediated phosphorylation of c-Jun. The immediate early genes of the AP-1 family and NF κ B may be chronically upregulated during disease circumstances such as asthma, leading to a continued production of pro-inflammatory cytokines. If this upregulation persists, then it is possible that the repressive effects of glucocorticoids may be overridden. Progress has been made in understanding the mechanisms of transcriptional repression by glucocorticoids. With the recently described production of dissociated ligands, it is possible that specific mechanisms of action of GR may be differentially targeted in order to fine tune therapeutic intervention. Additionally, it is likely that additional targets for GR regulation will be identified providing further targets for pharmaceutical action.

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